# Design and Synthesis of Novel 1-substituted-3-(3-(3-nitrophenyl)-4-oxo-3,4-dihydrobenzopyrimidin-2-yl amino) Isothioureas for their Anti-HIV, Antibacterial Activities, Graph Theoretical Analysis, Insilico Modeling, Prediction of Toxicity and Metabolic Studies

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Key words
condensed pyrimidine, graph theoretical analysis, substituted thiosemicarbazide, antibacterial activity, antitubercular activity, anti-HIV activity

**received** 30.06.2019
**accepted** 05.08.2019

Bibliography
**DOI** https://doi.org/10.1055/a-0991-7617
Published online: 19.6.2020
Drug Res 2020; 70: 348–355

ISSN 2194-9379

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**Supporting Information** for this article is available online at http://www.thieme-connect.de/products

**ABSTRACT**

In the present study, we have placed the substituted thiosemicarbazide moiety at the C-2 position and 3-nitrophenyl group at N-3 position of benzopyrimidines and studied their antitubercular, anti-HIV and antibacterial activities against selected gram positive and negative bacteria. The target compounds 1-substituted-3-(3-(3-nitrophenyl)-4-oxo-3,4-dihydrobenzopyrimidin-2-ylamino) isothioureas (**PTS1 – PTS15** ) were obtained by the reaction of 2-hydrazino-3-(3-nitrophenyl) benzopyrimidin-4(3 H)-one (**5**) with different alkyl/aryl isothiocyanates followed by methylation with dimethyl sulphate. All synthesized compounds were screened for their antitubercular, anti-HIV and antibacterial activity against selective gram positive and gram negative bacteria by agar dilution method. Among the series, compound 2-methyl-3-(3-(3-nitrophenyl)-4-oxo-3,4-dihydrobenzopyrimidin-2-ylamino)-1-(3-chlorophenyl)isothiourea **(PTS14)** shown most potent activity against *Klebsiella pneumoniae*, *Proteus vulgaris* and *Staphylococcus aureus*; **PTS14** exhibited the antitubercular activity at the minimum microgram of 1.56 µg/mL and anti-HIV activity at 0.96 µg/mL against HIV1 and HIV2 and offers potential for further optimization and development to new antitubercular and anti-HIV agents. The results obtained from this study confirm that the synthesized and biologically evaluated benzopyrimidines showed promising antimicrobial, antitubercular and anti-HIV activities and are new scaffolds for antimicrobial activity.

## Introduction

Tuberculosis (TB) is an opportunistic infection that occurs more often or is more severe in people with weakened immune systems than in people with healthy immune systems. Human immunodeficiency virus (HIV) weakens the immune system, increasing the risk of TB in people with HIV. Co-infection with TB and HIV poses a tremendous challenge to TB control, particularly in resource-limited treatment option. In 2015, it was estimated 10.4 million cases of TB disease, among that 1.2 million (11 %) people living with HIV, there were an estimated 456 000 deaths from HIV-associated TB [1–4]. Although exhaustive efforts to prevent and treat TB is taken the problem still continues due to multi-drug-resistant (MDR-TB) to isoniazid, rifampicin, quinolones and aminoglycosides. Recently TB threat has an additional challenge with the emergence of both multi-drug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) strains. It clearly highlights the urgent need to devel-

op novel "druggable" molecules for the co-infection treatment and strains of MDR-TB and XDR-TB.

Recent year's pyrimidines and condensed pyrimidines gained much attention to medicinal chemists and pharmacologist due to their potential druggable behaviour [5]. Among that antimicrobial activities of 2,3-disubstituted benzopyrimidines are encouraging for further development. Recent literature evident that the 2,3-disubstituted benzopyrimidines nucleus showed significant antitubercular activity [5, 6].

Thiosemicarbazones have been explored for various medicinal chemistry properties due to their widespread pharmacological activities including antineoplastic, antimycobacterial, antibacterial, antifungal, antiviral, and antimalarial. Thiosemicarbazones bind great coordination with target sites because of its versatility due to nitrogen and sulfur atoms. Thiosemicarbazone is also known as an iron-chelating agent, bonding the sulfur and azomethine nitrogen atoms. The complexes of nitrogen and sulfur atoms with metal ions may be considered prospective pharmacological behaviour. In recent literature, many thiosemicarbazone derivatives have been synthesized and evaluated for their antibacterial activity. The current use of these agents in bacterial infections has led to the development of novel antibacterial and antitubercular agent [7–15].

The present work is a continuation of our efforts towards developing potent antitubercular and antimicrobial agents by a pharmacophore hybrid approach using the benzopyrimidine scaffold. In this approach a hybrid molecule was created by merging two or more pharmacophores. Therefore, a hybrid molecule containing more than one pharmacophore, each pharmacophores may be addressing the active site of targets and offer the opportunity to selectivity, further it can also reduce unwanted side effects [16]. In the present study, we have placed the substituted phenyl moiety at the N-3 position and substituted thiosemicarbazide group at C-2 position of benzopyrimidines ring ▶ **Supporting Information Fig. 1S and** ▶ **Fig. 1** [16–18] and studied their anti-HIV, antitubercular and antibacterial activities against selected gram positive and negative bacteria.

## Materials and Methods

### General

The molecular docking of test compounds was studied by using Schrodinger, LLC, New York, version 11 (2016) GLIDE program. In open capillaries melting points (mp) were measured on a Thomas Hoover melting point apparatus (Thomas Hoover, USA) and are uncorrected. Using potassium bromide disks the IR spectra (v, $cm^{-1}$) were recorded on Bruker FT-IR spectrometer (Bruker, USA). Using Bruker FT-NMR spectrometer (Bruker, USA) the $^1$H-NMR spectra were recorded in $CDCl_3$ at 300 MHz. The chemical shifts are reported as parts per million (δ, ppm) using tetramethylsilane (TMS) as an internal standard. Using fast atom bombardment (FAB positive) mass spectra were obtained on a JEOL-SX-102 instrument (JEOL, Japan). Perkin Elmer 2400 CHN analyzer (Perkin Elmer, USA) was used to perform elemental analysis and values were within the acceptable limits of the calculated values (± 0.4 %). Readymade silica gel plates (Merck, Norway) are used to monitor the progress of the reaction. All chemicals and reagents used in the synthesis were obtained from Merck, SD fine chemicals, Aldrich (USA), Lancaster (USA), or Spectrochem (India) and were used without further purification.

### General procedure for the synthesis of compound

#### Synthesis of 3-(3-nitrophenyl)-2-thioxo-2,3-dihydro benzopyrimidin-4-one **(3)**

A solution of the 3-nitro aniline (0.02 mol) in dimethyl sulfoxide (10 mL) was stirred vigorously. Carbon disulphide (1.6 ml) and aqueous 20 M sodium hydroxide (1.2 mL) was added drop wise to the above 3-nitro aniline solution during 30 min. Gradually dimethyl sulphate (0.02 mol) was added by keeping the reaction mixture stirring in freezing mixture for 2 h. The obtained reaction mixture was poured into ice cold water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol.

To the above prepared N-(3-nitrophenyl)-methyl dithiocarbamic acid (0.02 mol), methyl anthranilate (0.02 mol) was added and dissolved in ethanol (20 mL). Anhydrous potassium carbonate (100 mg) was added to the above mixture and refluxed for the 22 h. Using ice the reaction mixture was cooled and the solid separated was filtered. The obtained product (**3**) was purified by dissolving in 10 % alcoholic sodium hydroxide solution and re-precipitated by treating with dilute hydrochloric acid. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Yield: 86 %; mp: 281–282 °C; IR (KBr) $cm^{-1}$: 3355 (NH), 3062 (Ar-CH), 1729 (C = O), 1607 (C = C), 1520 & 1346 ($NO_2$), 1251 (C = S); $^1$H NMR ($CDCl_3$): δ6.73–6.75 (m, 2 H, Ar-H), 7.26 (d, J = 7.5 Hz, 1 H, Ar-H), 7.52–7.54 (m, 2 H, Ar-H), 7.71 (d, J = 2.0 Hz, 1 H, Ar-H),7.95 (d, J = 7.5 Hz, 1 H, Ar-H), 8.01 (d, J = 2.0 Hz, 1 H, Ar-H), 8.09 (m, 1 H, Ar-H),9.16 (brs, 1 H, CSNH); MS (m/z): 299 [$M^+$]; Anal. Calcd. for($C_{14}H_9N_3O_3S$): C, 56.18; H, 3.03; N, 14.04. Found: C, 56.31; H, 3.02; N, 14.00.

#### Synthesis of 2-methylthio-3-(3-nitrophenyl) benzopyrimidin-4-one **(4)**

The 3-(3-nitrophenyl)-2-thioxo-2,3-dihydro benzopyrimidin-4-one (**3**) (0.01 mol) was dissolved in alcoholic sodium hydroxide solution (25 mL). To this dimethyl sulphate (0.01 mol) was added drop wise with stirring and stirring was continued for 1 h after complete addition of dimethyl sulphate. The reaction mixture was then poured into ice cold water. The solid obtained was filtered, washed with water, dried and recrystallized from ethanol. Yield: 91 %; mp: 181–182 °C; IR (KBr) $cm^{-1}$: 3059 (Ar-CH), 2957 ($CH_3$-CH), 1715 (C = O), 1668 (C = N), 1612 (C = C), 1534 & 1326 ($NO_2$), 672 (C-S-C); $^1$H NMR ($CDCl_3$) δ: 2.20 (s, 3 H, $SCH_3$), 7.08–7.10 (m, 2 H, Ar-H), 7.26 (d, J = 7.5 Hz, 1 H, Ar-H), 7.52–7.54 (m, 2 H, Ar-H), 7.71 (d, J = 2.0 Hz, 1 H, Ar-H), 7.90 (d, J = 7.5 Hz, 1 H, Ar-H), 7.92 (d, J = 2.0 Hz, 1 H, Ar-H); MS (m/z): 313 [$M^+$]; Anal. Calcd. for($C_{15}H_{11}N_3O_3S$): C, 57.50; H, 3.54; N, 13.41. Found: C, 57.67; H, 3.55; N, 13.38.

#### Synthesis of 2-hydrazino-3-(3-nitrophenyl) benzopyrimidin-4-one **(5)**

The 2-methylthio-3-(3-nitrophenyl) benzopyrimidin-4-one (**4**) (0.01 mol) was dissolved in ethanol (25 mL). To this mixture 99 % hydrazine hydrate (0.1 mol) and anhydrous potassium carbonate (100 mg) was added and refluxed for 33 h. The reaction mixture was cooled and poured into ice cold water. The solid so obtained

was filtered, washed with water, dried and recrystallized using ethanol to afford the compound (**(5)**. Yield: 83 %; mp: 200–201 °C; IR (KBr) $cm^{-1}$: 3371 & 3286 (NH), 3035 (Ar-CH), 1748 (C = O), 1650 (C = N), 1621 (C = C), 1519 & 1353 ($NO_2$); $^1H$ NMR ($CDCl_3$) δ**:** 5.72 (brs, 1 H, Ar-NH), 6.17 (brs, 2 H, $NH_2$), 6.73–6.75 (m, 2 H, Ar-H), 6.94 (d, J = 7.5 Hz, 1 H, Ar-H), 7.42–7.44 (m, 2 H, Ar-H), 7.61 (d, J = 2.0 Hz, 1 H, Ar-H), 7.95 (d, J = 7.5 Hz, 1 H, Ar-H), 8.18 (d, J = 2.0 Hz, 1 H, Ar-H); MS (m/z)**:** 297 [$M^+$]; Anal. Calcd. for ($C_{14}H_{11}N_5O_3$): C, 56.56; H, 3.73; N, 23.56. Found: C, 56.45; H, 3.75; N, 23.64.

### Synthesis of 3-(3-nitrophenyl)-2-(substituted thiosemicarbazido) benzopyrimidin-4-one (**6A–6O**)

A mixture of compound 5 (0.01 mol) and alkyl/ aryl isothiocyanate (0.01 mol) in dioxane (25 mL) was refluxed for 6 h. The reaction mixture was concentrated and the product obtained was filtered, dried and recrystallised from dioxane.

Refer details of **6A–6O** experimental characterization in Supporting Information.

### 1-Substituted-3-(3-(3-nitrophenyl)-4-oxo-3,4-dihydrobenzopyrimidin-2-ylamino) isothioureas (**PTS1 – PTS15**)

The compound **6** (0.01 mol) was dissolved in alcoholic sodiumhydroxide solution (20 mL; 0.01 mol). To this mixture dimethyl sulphate (0.01 mol) was added drop wise with stirring. Stirring was continued for 3 h and poured in to ice water. The solid obtained was filtered, dried and recrystallized from ethanol.

Refer details of **PTS1–PTS15** experimental characterization in Supporting Information.

## Pharmacology

### Antitubercular activity

Into Middle brook 7H11 agar slants 10 fold serial dilutions of each test compound/drug were incorporated with OADC growth supplement. Fresh Middle brook 7H11 agar slants with OADC growth supplement was used to prepare inoculums of M. tuberculosis $H37R_V$ and adjusted to 1 mg/mL in Tween 80 (0.05 % W/V) saline diluted to $10^{-2}$ to give a $10^7$ cfu/mL concentrate approximately. Into 7H11 agar tubes containing 10 fold serial dilutions of drug per ml a 5 µL amount of bacterial suspension was spotted. At 37 °C the tubes were incubated, and after 28 days the final readings were recorded. Control tubes where medium alone was incubated with $H37R_V$ were used to compare tubes having the compounds. Active concentration of test compound was taken as the concentration at which complete inhibition of colonies occurred. The minimum concentration of compound required to give complete inhibition of bacterial growth was taken as MIC [19–21]. Against reference drug isoniazide, rifampicin and etahmbutol, the MIC of the test compounds was compared.

### Anti-HIV activity

In MT-4 cells anti-HIV activity of the compounds (**PTS1 – PTS15**) were tested against replication of HIV-1 (III B) and HIV-2 (ROD) [22]. The MT-4 cells were grown in RPMI-1640 DM (Dutch modification) medium (Flow laboratories, Irvine, Scotland), supplemented with 10 % (v/v) heat inactivated fetal calf serum and 20 µg/mL gentamicin (E. Merck, Darmstadt, Germany). From the culture supernatant of HIV-1 infected MT-4 cell lines HIV-1 (III B) and HIV-2 (ROD) were obtained and the virus stocks were stored at − 70 °C until used. Microtiter plates was used to perform antiHIV assay by filled with 100 µL of medium and 25 µL volumes of compounds in triplicate so as to allow simultaneous evaluation of their effects on HIV and mock infected cells. 50 µL of HIV at 100 $CCID_{50}$ medium was added to either infected or mock infected part of microtiter tray. At 37 °C the cell cultures were incubated in a humidified atmosphere of 5 % $CO_2$ in air. By the MTT method after five days of infection spectrophotometrically examined the viability of mock and HIV infected cells. The effective dose of compound achieving 50 % protection of MT-4 cells against the cytopathic effect (Virus cause cell degeneration or cell death, which can be seen by microscopical examination of cultures. Cell degeneration is manifested by certain pathological changes) of HIV ($EC_{50}$) and the cytotoxic dose of compound, required to reduce the viability of normal uninfected MT-4 cells by 50 % ($CC_{50}$) were calculated.

### Antibacterial activity

Agar dilution method was used to evaluate antibacterial activity of compounds [23, 24]. From the American type culture collection (ATCC), Rockville, USA the standard strains were procured and from the department of microbiology, MNR medical college, Sangareddy, India the pathological strains were procured. The antibacterial activity of the test analogs was screened against the following bacterial strains: *E. coli* ATCC 25922, *P. vulgaris* ATCC 9484, *S. typhimurium* ATCC 33068, *K. pneumoniae* ATCC 13883, *P. aeruginosa* ATCC 2853, *B. subtilis* ATCC 6051, *S. aureus* ATCC25923, *M. luteus*ATCC 10240, *S. epidermidis* ATCC 35984, *S. albus* ATCC 17900. Muller–Hinton Agar (Hi-media) plates (37 °C, 24 h) were used for bacterial growth and the minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited the growth on agar plates, disregarding a single colony or faint haze caused by the inoculums. Ciprofloxacin was used as reference drug for comparison of the MIC of the test compounds. The values are mentioned in ▶**Table 1** calculated from at least three different experiments in duplicate.

### Graph theoretical analysis

Kyoto encyclopedia of genes and genomes database was utilized for the study of graph theoretical analysis. The influential proteins was recognized and chosen by network of protein interaction (mtu: 00770) in homo sapiens[25–28].

### Density functional theory

Gauss view molecular visualized program [29] and Gaussian 03 W package [30] was used to perform computational calculations of the test analogs. Using B3LYP functional with 6–311 G (d,p) basis set density functional theory (DFT) optimizes the ground state molecular structures of synthesized compounds. From the optimized structures the frontier molecular orbital energies [lowest unoccupied molecular orbital ($E_{LUMO}$), highest occupied molecular orbital ($E_{HOMO}$)and their energy gap (Eg)] of all molecules were derived. Gauss view molecular visualized program is used to visualize the obtained molecular orbital energy diagrams of the synthesized derivatives.

▲ **Table 1** Antitubercular, anti-HIV and antibacterial activity of title compounds (**PTS1 – PTS15**).

| Microorganism | MIC of test compounds / standard * (in µg/mL) | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PTS 1 | PTS 2 | PTS 3 | PTS 4 | PTS 5 | PTS 6 | PTS 7 | PTS 8 | PTS 9 | PTS 10 | PTS 11 | PTS 12 | PTS 13 | PTS 14 | PTS 15 | STD 1 | STD 2 | STD 3 |
| MTB | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 12.5 | 6.25 | 25 | 25 | 1.56 | 25 | 0.05 | 0.1 | 1.56 |
| HIV-1 | 44.54 | 45.28 | 47 | 39.52 | 48.51 | 49.66 | 100 | 100 | 43.60 | 8.28 | 2.91 | 9.95 | 9.42 | 0.96 | 10.58 | 0.0012 | – | – |
| HIV-2 | 44.54 | 45.28 | 47 | 33.94 | 48.51 | 49.66 | 100 | 100 | 43.60 | 8.28 | 2.91 | 9.95 | 9.42 | 0.96 | 10.58 | 0.000642 | – | – |
| S. typhi | 50 | 50 | 50 | 50 | 25 | 50 | 50 | 50 | 50 | 25 | 25 | 50 | 25 | 12 | 25 | 4 | – | – |
| E. coli | 50 | 50 | 25 | 25 | 50 | 50 | 50 | 100 | 50 | 25 | 12 | 50 | 25 | 12 | 25 | 1 | – | – |
| B. subtilis | 25 | 25 | 25 | 25 | 25 | 50 | 50 | 50 | 25 | 12 | 12 | 12 | 12 | 6 | 25 | 1 | – | – |
| K. pneumoniae | 25 | 25 | 50 | 25 | 50 | 50 | 100 | 50 | 25 | 6 | 6 | 12 | 12 | 1.6 | 12 | 1 | – | – |
| P. vulgaris | 50 | 50 | 50 | 25 | 50 | 50 | 50 | 50 | 50 | 12 | 1.6 | 1.6 | 12 | 1.6 | 25 | 1 | – | – |
| P. aeruginosa | 50 | 50 | 50 | 25 | 50 | 50 | 100 | 100 | 50 | 25 | 12 | 12 | 12 | 12 | 12 | 1 | – | – |
| S. aureus | 50 | 50 | 50 | 50 | 50 | 50 | 100 | 100 | 50 | 6 | 6 | 12 | 13 | 1.6 | 25 | 1 | – | – |
| M. luteus | 50 | 50 | 50 | 25 | 50 | 50 | 50 | 50 | 50 | 12 | 12 | 12 | 12 | 6 | 12 | 1 | – | – |
| S. epidermidis | 50 | 50 | 50 | 50 | 50 | 50 | 100 | 100 | 50 | 12 | 6 | 25 | 25 | 6 | 25 | 1 | – | – |
| S. albus | 50 | 50 | 50 | 50 | 50 | 50 | 100 | 100 | 50 | 6 | 12 | 25 | 25 | 12 | 12 | 1 | – | – |

Antitubercular standard: STD 1 – Isoniazid, STD 2 – Rifampicin, STD 3 – Ethambutol; Anti-HIV standard: STD 1 – AZT; Antibacterial standard: STD 1 – Ciprofloxacin. – Not determined

### Molecular docking

GLIDE program is used to perform the molecular docking studies of compounds. Based on the report of network analysis 3IVX was chosen as the target proteins. In this study the research collaboratory for structural bioinformatics (RCSB) protein data bank was used to retrieve the X-ray crystal structures of target proteins 3IVX [Pantothenatesynthetase (panC)] along with literature based target proteins 1BVR [2-trans-enoyl-ACP reductase (InhA)] and 3KRD [Proteasome]. Extra precision (XP) mode of GLIDE program is used for performing all docking calculations. GLIDE score (G score) is used to select the greatest docked structure.

### ADME & Toxicity studies

In clinical trials poor absorption, distribution, metabolism, and excretion (ADME) properties is the major reason for failing of most of drug candidates. Thus avoiding of compounds not having drug likeliness and good ADME property is an important aspect of drug discovery. The QikProp module of Schrodinger is a quick and accurate tool so that its easy-to-use to identify ADME prediction program design to produce certain descriptors related to ADME. Both physicochemical significant descriptors and relevant properties of pharmacokinetics are predicted by the QikProp module. Based on Lipinski's rule of five ADME properties determines the drug-like activity of ligand molecules. Qikprop module of Schrodinger 2016 (https://www.schrodinger.com/qikprop) was used to study the ADME/T property of the test compounds.

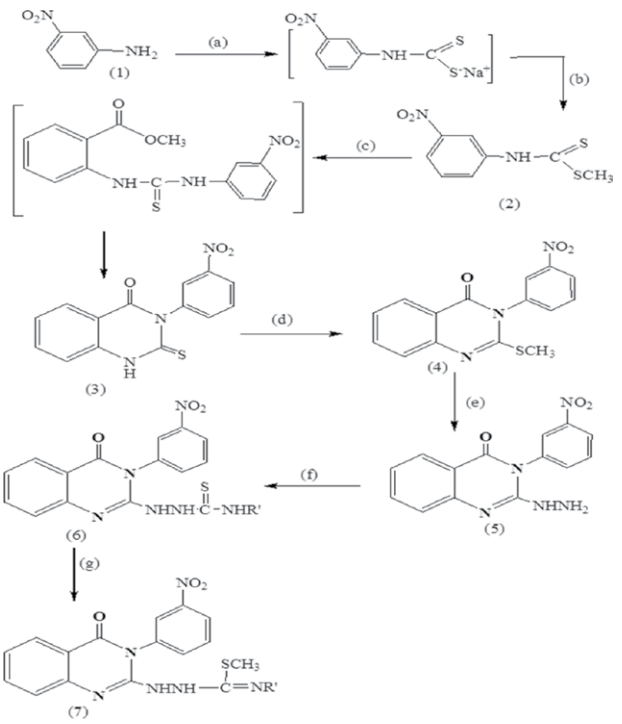
### SOM prediction using SMARTCyp

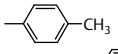
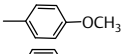
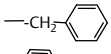
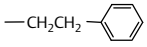
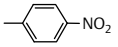
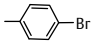
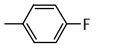
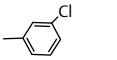
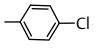
SMARTCyp software is a SOM predictor, released by Rydberg [31]. The drug molecule was uploaded in SMILES strings format representing the molecule. SMARTCyp contains a database of ligands metabolized by CYP450 and energies were pre-calculated by the activation of DFT. The database contains smiles arbitrary, target specification based fragments and it was used by SMARTCyp combination with an accessibility descriptor to obtain a ranking of SOM.

## Results and Discussion

### Chemistry

By using new innovative route the key intermediate 3-(3-nitrophenyl)-2-thioxo-2,3-dihydro-1H- benzopyrimidin-4-one (**3**) was prepared (▶ **Fig. 1**). In which 3-nitro aniline (**1**) treated with carbon disulphide and sodium hydroxide in dimethyl sulphoxide to give sodium dithiocarbamate, which was methylated with dimethyl sulphate to afford the dithiocarbamic acid methyl ester. The dithiocarbamic acid methyl ester on reflux with methyl anthranilate **(2)** in ethanol yielded the desired 3-(3-nitrophenyl)-2-thioxo-2,3-dihydro-1H-benzopyrimidin-4-one **(3)** via the thiourea intermediate in good yield (86 %). Rate of reaction is enhanced by the use of DMSO as the reaction solvent and due to less solvation hydrolysis of the intermediate are prevented by the use of alkali in higher concentration. The product obtained was cyclic and not an open chain thiourea. The IR spectrum of **3** show intense peaks at 3355 $cm^{-1}$ for cyclic thiourea (NH), 1729 $cm^{-1}$ for carbonyl (C = O) and 1251 $cm^{-1}$ for thioxo (C = S) stretching. $^1H$ NMR spectrum of **3** showed multiplet due to aromatic (8 H) protons at δ 6.73–8.09 ppm and a sin-




| Compound | -R | Compound | -R | Compound | -R |
|---|---|---|---|---|---|
| PTS 1 | $-CH_3$ | PTS 2 | $-C_2H_5$ | PTS 3 | $-CH_2CH_2CH_3$ |
| PTS 4 | $-CH_2CH=CH_2$ | PTS 5 |  | PTS 6 | $-C_6H_5$ |
| PTS 7 |  | PTS 8 |  | PTS 9 |  |
| PTS 10 |  | PTS 11 |  | PTS 12 |  |
| PTS 13 |  | PTS 14 |  | PTS 15 |  |

▶ **Fig. 1** Synthesis of 1-substituted-3-(3-(3-nitrophenyl)-4-oxo-3,4-dihydrobenzopyrimidin-2-ylamino)isothioureas. Reagents and conditions: **a** $CS_2$, NaOH, DMSO, 30 min; **b** Dimethyl sulphate, 2 h; **c** Methyl anthranilate, Anhydrous $K_2CO_3$, EtOH reflux, 22 h; **d** 2 % alcoholic sodium hydroxide solution. Dimethyl sulphate, 1 h; **e** Hydrazine hydrate, Anhydrous potassium carbonate, Ethanol reflux, 33 h; **f** alkyl/aryl isothiocyanates, Ethanol reflux, 6 h; **g** NaOH, Dimethyl sulphate.

glet at δ 9.16 ppm indicating the presence of NH. Data from the elemental analyses have been found to be in conformity with the assigned structure. Further, the molecular ion recorded in the mass spectrum is also in agreement with the molecular weight of the compound.

The 2-methylsulfanyl-3-(3-nitrophenyl)-3H-benzopyrimidin-4-one **4** was obtained by dissolving **3**in 2 % alcoholic sodium hydroxide solution and methylating with dimethyl sulphate with stirring at room temperature. The IR spectrum of **5** showed disappearance of NH and C = S stretching signals of cyclic thiourea. It showed a peak for carbonyl (C = O) stretching at 1715 $cm^{-1}$. The $^1H$ NMR spectrum of compound **4** showed singlet at δ 2.20 for $SCH_3$ group; and a multipletfor aromatic (8 H) protons observed at δ 7.08–7.92 Data from the elemental analyses and molecular ion recorded in the mass spectrum further confirmed the assigned structure.

Nucleophilic displacement of $-SCH_3$ group of **4** with hydrazine hydrate was carried out using ethanol as solvent to afford 2-hydrazino-3-(3-nitro phenyl)-3H-benzopyrimidine **5**. The long duration of reaction (33 h) required might be due to the presence of bulky butyl group at position **4**, which might have reduced the reactivity of quinazoline ring system at C-2 position. The formation of **5** was confirmed by the presence of NH and $NH_2$ signals at 3371–3286 $cm^{-1}$ in the IR spectrum. It also showed a peak for carbonyl (C = O) at 1748 $cm^{-1}$. The $^1H$ NMR spectrum of the compound **5** showed singlets δ 5.72 and 6.17 ppm due to $NH_2$ and NH respectively, a multiplet at δ 6.94–8.18 ppm was observed for aromatic (8 H) protons. Data from the elemental analyses have been found to be in conformity with the assigned structure. Further the molecular ion recorded in the mass spectrum is also in agreement with the molecular weight of the compound.

The compounds 1-(3-(3-nitrophenyl)-4-oxo-3H-dihydroquinazolin-2-yl)-4-(substituted) thiosemicarbazides (**6A – 6O**) were obtained by the condensation of amino group of 3-(3-nitrophenyl)-2-hydrazino-3H-benzopyrimidin-4-one **(5)** with a variety of alkyl/aryl isothiocyanates. The formation of desired products is indicated by the disappearance of peak due to NH, $NH_2$ of the starting material in IR and $^1H$ NMR spectrum of all the compounds **6A–6O**. The IR and $^1H$ NMR spectrum of these compounds showed the presence of peaks due to thiosemicarbazides, carbonyl (C = O), NH and aryl groups. Data from the elemental analyses have been found to be in conformity with the assigned structure. The mass spectrum is also in agreement with the molecular weight of the compound.

The title compounds 1-substituted-3-(3-(3-nitrophenyl)-4-oxo-3,4-dihydrobenzopyrimidin-2-ylamino)isothioureas (**PTS1 – PTS15**,) were obtained by the methylation of the thiosemicarbazides (**6A–6O**) using dimethyl sulphate. The formation of title product is indicated by the disappearance of peak due to NH and C = S of the starting material in IR and $^1H$ NMR spectrum of all the compounds **PTS1 – PTS15**. The IR and $^1H$ NMR spectrum of these compounds showed the presence of peaks due to methylthiosureas, carbonyl (C = O), NH and aryl groups. The mass spectra of the title compounds showed molecular ion peaks corresponding to

their molecular formulae. In mass spectra of compounds **PTS1 – PTS15**, a common peak at m/z 144 corresponding to quinazolin-4-one moiety appeared in all mass spectrum of the compounds. Elemental (C, H, N) analysis satisfactorily confirmed elemental composition and purity of the synthesized compounds.

## Antitubercular activity

Against *M. tuberculosis* strain $H37R_V$ the synthesized compounds (▶ **Table 1**) were screened for their *in vitro* antimycobacterial activity. The results are expressed in terms of Minimum Inhibitory Concentration (MIC). The results of antimycobacterial activity depicted in ▶ **Table 1**, indicates that the test compounds inhibited the growth of *Mycobacterium* in varying degree. Compounds with aliphatic substituents showed lesser antitubercular activity over the aryl and heteroaryl substituents. The compounds with electron withdrawing substituent on the aryl ring showed better activity over the unsubstituted or electron donating substituent on the aryl ring. Among the test compounds, 2-methyl-3-(3-(3-nitrophenyl)-4-oxo-3,4-dihydrobenzopyrimidin-2-ylamino)-1-(3-chlorophenyl) isothiourea **(PTS14)** exhibited most potent antitubercular activity at the minimum microgram (1.56 µg/mL) concentration.

## Anti-HIV activity

Mild to moderate anti-HIV activity was exhibited by all compounds; whereas compounds **PTS14** containing electron withdrawing groups exhibited anti-HIV activity at 0.96 µg/mL against HIV1 and HIV2. Compounds with aliphatic substituents showed lesser antitubercular activity over the aryl substituted (expect **PTS9** and **PTS10)**. Aryl compounds with electron withdrawing substituents like chloro and nitro showed better activity over the unsubstituted. While the test compounds with other substituent's showed moderate anti-HIV activity against HIV1 and HIV2 with the MIC in the range of 2.91 to 100 µg/mL.

## Antibacterial activity

Among the different substituents at N-3 position of the quinazolin-2-yl, aryl substituents exhibited better activity over the aliphatic cyclic substituents (▶ **Table 1**). Compounds with electron withdrawing substituents like chloro and nitro showed better activity over the unsubstituted and electron donating substituents. Among the series, compound 2-methyl-3-(3-(3-nitrophenyl)-4-oxo-3,4-dihydrobenzopyrimidin-2-ylamino)-1-(3-chlorophenyl) isothiourea **(PTS14)** shown most potent activity against *K. Pneumonia P. Vulgaris and S. aureus*; while the compound **PTS11** and **PTS12** showed most potent activity against *P. Vulgaris*. Compounds **PTS14, PTS11** and **PTS 12** were emerged as the most active compounds of the series.

## Graph theoretical analysis

The pathway was renewed to a graph with proteins as nodes and interactions as edges and it was represented in ▶ **Supporting Information Figs. 2S** and **3S**, ▶ **Tables 1S** and **2S**. The network was carries 61 nodes and 68 edges. Parameters like degree centrality, betweenness centrality, eigenvector centrality, radialitycentrality, stress centrality and closeness centrality from the network was calculated to identify the significance of proteins. The target was identified as pan C based on its threshold values. In addition pan C has more attention because of its interaction between TB proteins which is cause of spreading disease. From the study it was found that pan C is a significant target for TB to identify active quinazoline moiety.

## Density functional theory

In bioactivities the frontier-orbital energies of a compound play an important role. $E_{HOMO}$ is one among that which is a rough measure of electron donating ability of a compound. In general, biological activity is increasing with increased value of $E_{HOMO}$, whereas the $E_{LUMO}$ acts in reverse. Chemical reactivity of the molecule and bioactivity of compounds is reflected by energy values of the $E_{LUMO}$ and $E_{HOMO}$ and their ΔE. $E_{HOMO}$ energy falls over at the **PTS7** of parent ring but $E_{LUMO}$ energy falls on parent **PTS14** in an equal ratio to $E_{HOMO}$ value, hence for all the synthesized compounds energy gap value are in the same range and there is no much difference ▶ **Supporting Information Table 3S**. Results suggests substitution of electron withdrawing group at parent ring **PTS11 – PTS15** can further increase $E_{HOMO}$ energy and reduce the $E_{LUMO}$ energy, thus there was an increase in energy gap and it turns the stability and bioactivity of compounds.

## Molecular docking

In the G-score form the scoring function of GLIDE docking program is presented. The most straight forward method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose (binding conformation) predicted by the object scoring function. In this study, from the G-score by removing the inhibitor compound with selected proteins has been analyzed for validation of extra precision GLIDE docking procedure. In ▶ **Supporting Information Table 4S** the docking result of these ligands are summarized. For each minimized complex the interaction energy includes van der Waals energy, electrostatic energy, as well as intermolecular hydrogen bonding were calculated and presented in ▶ **Supporting Information Tables 4S** and **5S**. Towards panC protein all fifteen designed ligand molecules showed potent interaction. The docking score was ranging from −4.590 to −8.039 for the tested series **PTS1 – PTS15** with panC protein ▶ **Supporting Information Fig. 4S**. These synthesized analogs displayed low scoring against the literature based chosen other InhA and Proteasome targets. By hydrophobic and hydrophilic interaction all the test molecules bound to protein, which is shown in ▶ **Supporting Information Figs. 4S** and ▶ **5S**. From the in silico results, it was found that towards panC protein the designed test compounds showed potent and selective action. Thus it proves that, to find potential analogue towards antiTB drug development the network based target selection was effective and most significant. antiTB potency of the test analogs was proved form observed results. Among various tested analogs, the synthesized analogues **PTS 11 – PTS15** were found to be more potent and selective in action against panC protein.

## Ligand based toxicity prediction

QikProp module of Schrodinger uses toxicity properties to categorize the drug-like activity of the ligand molecule. QikProp module of Schrodinger was used to study the toxicity property of synthesized molecules and the obtained results were shown in ▶ **Support-**

**ing Information Table 6S**. Test compounds are considered to be drug like compound because it potentially satisfied Lipinski's rule of five. In addition all predicted properties of test analogs were in the range of 95 % of known oral drugs.

### SOM prediction using SMARTCyp

SMARTCyp was used to predict the test compounds primary site of metabolism. From the study it was found that test analogs undergoes metabolism preferentially by desulfonylation process. Moreover the metabolism was not takes place at substituted position of the test compounds ▶ **Supporting Information Fig. 6S**. From the SOM prediction study it was concluded that pharmacophore of the test moiety was not affected during metabolism.

## Conclusion

In summary, synthesis of new series of 1-substituted-3-(3-(3-nitrophenyl)-4-oxo-3,4-dihydrobenzopyrimidin-2-ylamino)isothioureas **PTS1 – PTS15** have been described. These derivatives have exhibited significant antibacterial activity against the various gram positive and gram negative bacteria including *M. tuberculosis*; and moderate activity against HIV1 and HIV2 strains. The substituents at the thiosemicarbazide shown varied antimicrobial activity, aryl substituents with electron withdrawing group showed most potent and the allyl, alkyl/aryl substituents showed moderate activity and the aryl substituents with electron donating groups showed the least activity, among the series, compound 2-methyl-3-(3-(3-nitrophenyl)-4-oxo-3,4-dihydrobenzopyrimidin-2-ylamino)-1-(3-chlorophenyl)isothiourea **(PTS14)** shown most potent activity against *K. Pneumonia P. Vulgaris* and *S. aureus*; **PTS14** exhibited the antitubercular activity at the minimum microgram (MIC: 1.56 µg/ml) and antiHIV activity at 0.96 µg/mL against HIV1 and HIV2 and offers potential for further optimization and development to new antitubercular and anti-HIV agents.

### Conflict of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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